Diminished Role of Dopamine D1-Receptor Signaling with the Development of an Addicted Phenotype in Rats

Supplemental Information

Supplementary Methods

Animals

Subjects were sexually mature intact male (*n* = 20) and female (*n* = 25), and ovariectomized (OVX) female (*n* = 51) Sprague-Dawley rats (Charles River) that were ~3 months old at the start of the study. OVX rats arrived at the research facility within 3 days of the OVX surgery, and were randomly assigned to either a vehicle-treated group (OVX+Veh, resistant) or to an estradiol-treated (OVX+E, vulnerable) group. Upon arrival, rats were group housed in a colony room during a 1-2 day habituation period. After habituation and for the remainder of the experiment, rats were individually housed in operant testing chambers (ENV-018M; Med Associates, St. Albans, VT). Rats were maintained on a 12-hr light/dark cycle (house-lights on at 7 AM) with ad libitum access to food and water except during cocaine self-administration training as detailed below. Rats were first pre-trained to lever press for sucrose pellets (45 mg) to ensure that cocaine self-administration was acquired rapidly using methods previously described (1). This brief training period was conducted under fixed-ratio 1 conditions (23 hr/day sessions) and ended following two consecutive sessions in which a rat obtained a minimum of 100 sucrose pellets. Health of rats was examined daily and weights were recorded three times/week. University of Virginia Animal Care and Use Committee approved all animal protocols which adhered to the guidelines set by the NIH.

Estradiol Replacement and Vaginal Cytology

Rats received subcutaneous injections (0.1 ml) of estradiol or sesame oil at 11:30 AM five days/week beginning within 5 days of the OVX surgery. This estradiol dose (5 μg/day) has been shown to result in physiologically relevant levels of estradiol (2), to enhance acquisition of cocaine self-administration (3), and to increase cocaine self-administration under extended access (ExA) conditions (4). Importantly, these estradiol versus vehicle conditions generated a vulnerable versus a resistant phenotype when responding was assessed following abstinence from ExA self-administration (4). Daily vaginal swabbing was used to confirm hormonal status using methods previously described (5). Swabs obtained from OVX+Veh were predominated by metestrus/diestrus-like cells and swabs from OVX+E rats were predominated by proestrus-like cells.

Surgery

Following at least 5 days of acclimation, rats were anesthetized and implanted with a chronic indwelling catheter using methods previously described (1,4-5). On the same day, rats were implanted with a bilateral stainless steel infusion cannula (23-gauge; Plastics One, Roanoke, VA) in the nucleus accumbens (NAc) core (coordinates: +1.2 mm, anterior to bregma; +/- 1.5 mm, lateral to midline; 5.7 mm, ventral to brain surface) using methods previously described (6-7). The core region of the NAc was targeted in this study because abundant information implicates this region in both early and late stages of the addiction process (8-10). To prevent blockage, dummy cannulas (Plastics One, Roanoke, VA) extending 1.0 mm beyond the guide cannula, were left in place throughout the experiment. Dental cement (Stoelting Co, Wood Dale, IL) and three superficial cranial screws (Plastics One, Roanoke, VA) were used to

affix the cannula to the skull. Cocaine self-administration training began following 5-6 days of recovery. Catheter patency was checked daily by flushing with a small amount of heparinized saline and then pulling back until blood appeared in the line. If a catheter was not patent (i.e., no blood appeared in the line), a new catheter was implanted into the left jugular vein, and testing resumed after a minimum of 2 days of recovery from surgery.

Cocaine Self-administration Procedures

Rats were trained to self-administer cocaine (1.5 mg/kg/infusion) using methods previously described (i.e., fixed-ratio 1 with a maximum of 20 infusions/day; 11). A relatively high dose of cocaine was used to ensure rapid rates of acquisition (4,11-12) and moderate food restriction (i.e., 16 g) was used briefly (2-3 days) when necessary. Acquisition was defined as the first two consecutive sessions in which a rat self-administered the maximum of 20 infusions available. All groups acquired rapidly under these high dose conditions and rates of acquisition did not differ between groups. Following acquisition, rats were randomly assigned to one of two access groups and were given 1) short access (ShA) to cocaine in which rats were maintained under the same fixed-ratio 1 schedule for three additional consecutive sessions, or 2) ExA to cocaine using methods previously described (11). Briefly, rats had 24-hour access to cocaine (1.5 mg/kg/infusion) under a discrete trial procedure (10 minute trials, 4 trials/hr) for 10 days. Previous work has shown that these access and dose conditions produce high and dysregulated patterns of cocaine intake with limited toxicity in intact males and females and OVX females with and without estradiol replacement (4-5,12-18). Trials initiated every 15 minutes for a total of 10 days allowing for 4 infusions/hour and 96 infusions/24-hr period. Following the 10th day of discrete trial access, cocaine infusions were again available under a fixed-ratio 1 schedule

with a maximum of 20 infusions available/session for two consecutive sessions. These fixed-ratio sessions served as a control to minimize the predicted between-group differences in levels of intake during ExA conditions prior to abstinence. At the end of both ShA and ExA self-administration, rats remained in their operant testing chambers during a 14-day abstinence period with both levers retracted and only the house light illuminated from 7 AM to 7 PM.

Progressive-Ratio (PR) Cocaine Self-Administration

Following the 14th day of abstinence from ExA cocaine self-administration, a PR schedule was used to measure motivation for cocaine using methods previously described (19-20). Briefly, the ratio (i.e., number of lever presses) requirement to obtain an infusion increased progressively during the session. A modest-to-high cocaine dose 0.5 mg/kg/infusion was selected based on our recent work showing that this dose was sensitive to detecting a vulnerable versus a resistant phenotype following abstinence from ExA conditions (4). The effect of intra-accumbens infusion of SCH23390 on PR responding for cocaine began once baseline responding was established under the PR schedule. Baseline was defined as three consecutive sessions with no increasing or decreasing trend in the number of infusions obtained. Typically, the initial baseline was established within the first three sessions.

Site-specific Micro-infusions

The effects of SCH23390 (0.0, 0.3, 1.0, 3.0 μg/side) were examined using a within-subject design. These doses were selected from past studies (21-27) to include a moderate-to-high dose, a moderate-to-low dose, and a low-dose that modulate cocaine reinforcement maximally, moderately and ineffectively. Rats were randomly assigned to receive the different

doses of SCH23390 in a counterbalanced fashion. After determining baseline, rats received a two-minute bilateral micro-infusion (0.5 ul/side) immediately before the PR test session. To do so, the dummy cannulas were removed and injection cannulas (attached to tubing leading to an infusion pump) (Harvard Apparatus, S. Natick, MA) were manually placed in the cannula guides. Injectors were left in place for an additional two minutes to prevent diffusion up the guide cannulas. Rats were hand-held during all infusions and were then placed back in the operant chamber and the PR test session began. A stable baseline was established for each subject prior to each test session and a minimum of three stable sessions separated each test session. Due to experimental circumstances associated with this lengthy protocol, particularly the maintenance of catheter and cannula patency (i.e., rats typically received their first SCH23390 treatment ~40 days after the catherization surgery), not all animals received all four doses. Each animal received between 2 and 4 doses, with a minimum of 6 animals tested under each of the dose conditions.

Sucrose Self-administration

Additional groups of intact male (n = 5), female (n = 5), and OVX rats (OVX+E, n = 7; and OVX+Veh, n = 6) were tested on motivation to obtain sucrose pellets. These sucrose control rats were tested under the PR schedule following a 14-day abstinence period from sucrose self-administration under the fixed-ratio schedule using the same procedures as described above for ShA cocaine self-administration. The effects of D1-receptor antagonism were also tested using the same procedures described above.

Cannula Placement

Following the final micro-infusion treatment, coronal brain sections (2000 μm) were stained with methylene blue and cannula placement for each subject was verified and plotted on a histological diagram of the rat brain. For three subjects, placement was found to lie dorsal to the NAc in the striatum, and for one subject placement was determined to be in the NAc shell. These animals were removed from the study and are not included in the total numbers reported. For all other subjects, placement was within the NAc core (Figure S1).

Drugs

The D1-receptor antagonist SCH23390 was obtained from Sigma-Aldrich and was dissolved in sterile water. Cocaine hydrochloride was obtained from the National Institute on Drug Abuse. The same concentration (g/ml) was maintained throughout the study with mg/kg dose adjusted three times a week based on body weight (2 s/100 g body weight). β-Estradiol 3-benzoate was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in sesame oil.

Data Analysis

Separate analyses were conducted to examine differences between intact male versus female rats and OVX+E versus OVX+Veh rats. Intake under the ExA self-administration condition was compared between groups using a *t*-test. The number of cocaine infusions obtained during the first three stable PR sessions following abstinence from ExA versus ShA self-administration was compared between groups using repeated measures analysis of variance (ANOVA). The effect of SCH23390 on motivation for cocaine was examined by comparing the number of infusions obtained at baseline (averaged over the three sessions that preceded a test session) to those

obtained on the day of treatment using repeated measures ANOVA. Following a significant overall effect, comparisons were made within each group and within each dose using repeated measures ANOVA (baseline versus treatment). Its effects were also examined as percent change from baseline in the number of infusions obtained on the day of SCH23390 treatment in order to control for demonstrated baseline differences between groups (ShA versus ExA) using univariate ANOVA. Subsequent within group and dose comparisons were analyzed using univariate ANOVA. Posthoc comparisons to control or vehicle conditions were made using the Dunnett *t*-test, and comparisons within each day/dose were made using the *t*-test controlling for family-wise error. The same analyses were used to compare the effects of SCH23390 on responding for sucrose pellets. Statistical analyses were performed with IBM SPSS Statistics with alpha set at 0.05.

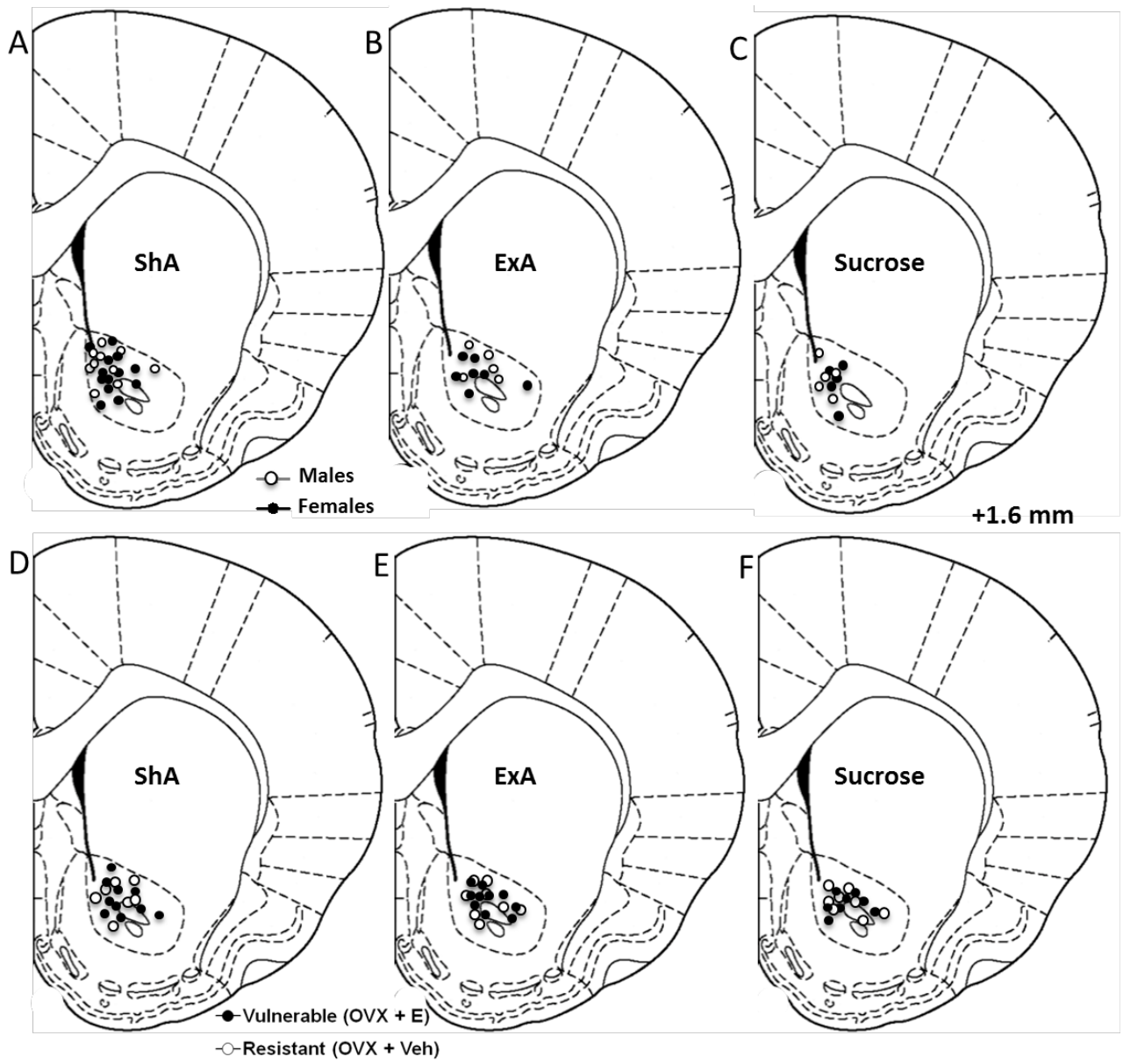

Figure S1. Micro-infusion site verification. Coronal section schematic representations of the site of the micro-infusions into the NAc (28) for each rat in the cocaine and sucrose groups. (**A-C**) Females, filled circles; Males, open circles. (**D-F)** OVX+E rats, filled circles; OVX+Veh rats, open circles. All micro-infusions were bilateral. E, estradiol; ExA, extended access; OVX, ovariectomized; ShA, short access; Veh, vehicle. Schematics from (28) reprinted with permission from Elsevier.

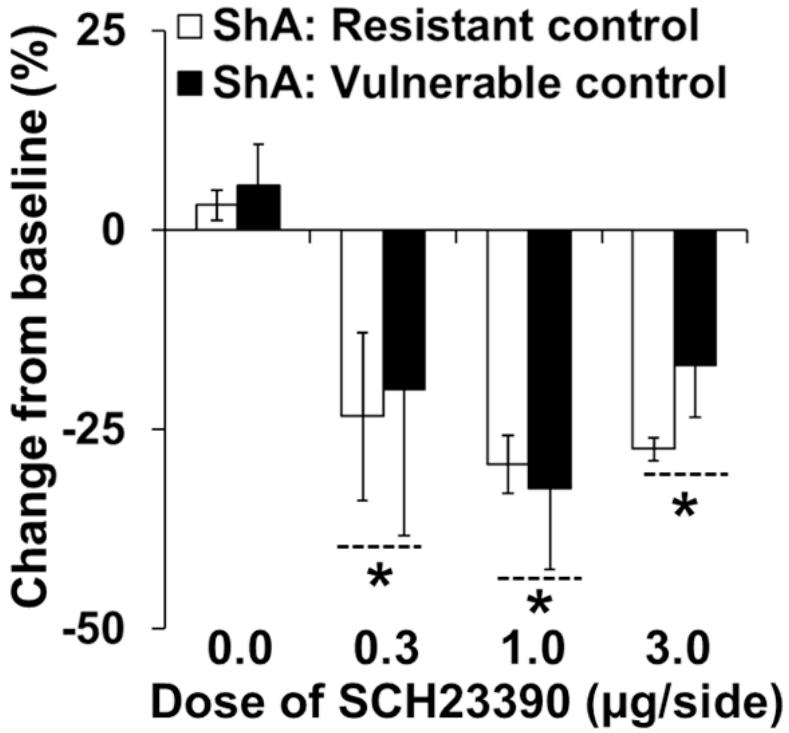

Figure S2. Comparison of the effect of SCH23390 on progressive-ratio responding for cocaine in the vulnerable versus the resistant short access (ShA) control groups. Mean (± SEM) percent change in number of infusions from baseline responding on the day of infusion of 0, 0.3, 1.0, or 3.0 µg of SCH23390. Open bars, ShA Resistant (*n* = 5-7); Black bars, ShA Vulnerable (*n* = 5-6) for each dose of SCH23390. *Significant difference from baseline.

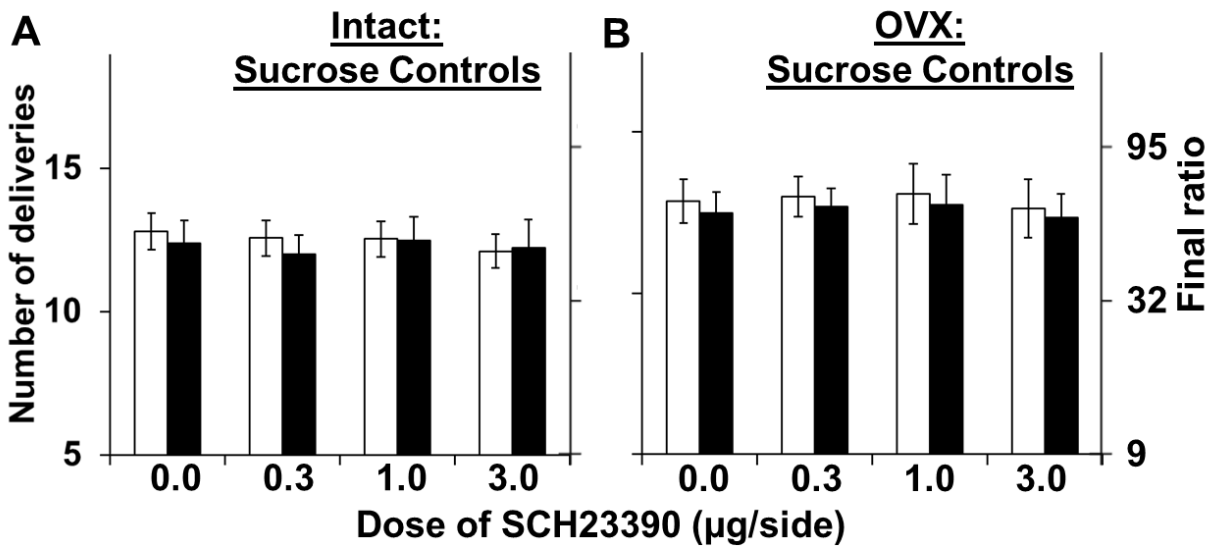

Figure S3. No effect of SCH23390 on motivation for sucrose. Mean (± SEM) number of sucrose pellet deliveries obtained and corresponding final ratios reached on a progressive-ratio schedule in (**A**) intact males and females and in (**B**) OVX+E and OVX+Veh rats at baseline (open bars) and on the day of SCH23390 treatment (filled bars; 0, 0.3, 1.0, and 3.0 µg). Male n = 5; Female n = 5; OVX+Veh n = 6-8; OVX+E n = 6-7 for each dose of SCH23390. OVX, ovariectomized.

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